VARIATION OF WEAK CATION-EXCHANGE POLYMER LAYERS GRAFTED ON HYDROPHOBIC MICROFILTRATION MEMBRANE FOR PROTEIN SEPARATION

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ABSTRACT

Preparation of material for protein separation can be done with controlled surface functionalizations on membranes. Photo-initiated surface-selective graft copolymerization was performed using entrapping method. Weak cation-exchange polymer brush structures were obtained using acrylic acid (AA) grafted on hydrophobic microfiltration membrane pore surfaces. Copolymerization of AA with "diluent" monomer acrylamide (AAm) and "cross-linker" monomer methylene bisacrylamide (MBAA) were done for variations of the grafted layer. Graft copolymer composition analysis had been performed using gravimetry. Performance characterizations had been done by measurements of membrane permeability at low and high pH and by reversible binding of a lysozyme. Result of this study shows that the membrane permeability sensitivity to changes in pH depending on type of modification, high with diluent and low with cross-linker. In addition to that, chemical cross-linking within grafted layers leads to a significant improvement because the dynamic protein binding capacity can be increased,

Key Words: Hyrophobic microfiltration membrane, photo-grafting, polymer brush, protein binding.

1.0 INTRODUCTION

Affinity chromatography technique permits the purification of proteins based on their surface charge, special domain structures or even their specific biological function [1]. Traditionally, packed beds are used, but this technology has several limitations. The high pressure drop across a packed bed, channelling due to uneven packing and, especially, the severe influence of slow intra-particle diffusion onto separation efficiency are the major problems. The latter effect causes also significant speed limitations for gradient elution, or complete buffer exchange and equilibration. All these problems make the scale-up of packed bed affinity chromatography or solid phase extraction difficult. Macroporous membranes had been proposed more than a decade ago in order to overcome the limitations of particle beds [1,2]. The transport of solutes through the membrane pores can take place by convection, the pressure drops for high flow rates are much lower, and the scale up is rather easy. In the meantime, first commercial membrane adsorbers are on the market. However, the interplay of membrane pore size and distribution, affinity binding and flow rates is still not understood in all details, and hence the potential of porous affinity membrane adsorbers can not be fully exploited yet.

Hydrophilic membranes have good characteristic of low non-specific adsorption of proteins but have poor thermal stability and are susceptible to chemical agents. In

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of Lys in the grafted polymer layers. However, not in all cases, this amount could be quantitatively recovered in the elution peak.

Focusing on eluted Lys amounts, the membranes with crosslinked grafted layer had higher values, and the AAc-AAm copolymer layers had values similar to the AAc homopolymer. Also, the recovery was significantly higher for the crosslinked AAc-MBAA copolymer membranes; in the second runs ~100% of the bound Lys could be recovered. Overall, an optimum of protein separation performance (highest binding and recovery) had been observed for the PP-g-PAAc-LMBAA membranes, i.e. the grafted layers with a low crosslinker content.

It should be noted, that under the evaluation conditions for protein binding, the membranes were in their stretched grafted layer conformation as deduced from the permeability measurements (cf. 3.2). Hence, as compared with the homopolymer brush membranes (PP-g-PAAc), the lower carboxyl content in the PP-g-PAAc-AAm membranes could be compensated by a higher degree of swelling allowing higher uptake; however, the release under elution conditions was not efficient enough. At an optimum crosslinker content (similar to PP-g-PAAc-LMBAA), the cation exchange polymer brush layers were more compact than the uncrosslinked layers with PAAc segments in linear chains, but still did not impose major accessibility limitations for the protein as could be observed for too high crosslinking degree (PP-g-PAAc-HMBAA).

4.0 CONCLUSION

Photo-initiated surface functionalization of porous polypropylene membranes using a mixture of an acrylamide crosslinker and AAc yielded formation of polymer network layers, while using a mixture of monofunctional acrylamide and AAc linear random copolymer brushes were obtained. Chemical crosslinking reduced the molecular mobility of the grafted brush layers and limits the swelling effects. As a consequence, the overall membrane performance is increased as compared with the linear structures. Therefore, at the same degree of functionalization and very similar composition, the surface layer architecture is the main factor to tailor membrane characteristics. Future work will focus on identification of suited functionalization conditions in order to optimise membrane adsorber properties and to gain a deeper understanding of structure / performance correlations.

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contrast, hydrophobic membranes have good thermal stability and chemical resistance but high non-specific protein adsorption. Therefore, a modification of hydrophobic polymer membranes that introduces hydrophilic segments on the surface is an ideal method for combining the advantages of hydrophilic and hydrophobic membranes [1].
Surface modification has become a key technology to improve the separation performance of already established membranes as well as to produce novel separation membranes. Photo-initiated graft copolymerization is a versatile approach to create chemically well-defined thin grafted polymer layers [3], e.g. on the entire internal surface of microfiltration membranes without damaging their matrix pore structure [4]. Such thin grafted polymer layers have impact onto the reduction of protein adsorption (fouling) [5]. Furthermore they can be used for the covalent immobilization of biomolecules [6]. One already established application of the latter approach is the affinity separation of proteins and other biomolecules by using porous affinity membrane adsorbers [6].

The aim of this work is to investigate systematically varied linear or crosslinked grafted functional polymer layers on the same porous polypropylene (PP) membrane by using the same functionalization. A new surface-selective photo-grafting method was used which is based on "entrapping" the photoinitiator in the surface layer of the PP membrane [7]. No initiator was added in the solution to avoid side reactions. In the present study, PP membranes with a cut-off pore size of ~0.4 μm were functionalized using acrylic acid, acrylamide and N,N'-methylene bisacrylamide. Water permeability as a function of pH and protein adsorption under membrane chromatography conditions were measured, and it was found that the architecture of grafted cation exchange polymer layers has indeed a great influence on the performance of the functionalized membranes.

2.0 EXPERIMENTAL

2.1 Materials

Polypropylene (PP) membranes (Accurel PP 2EHF, cut-off pore size ~0.4 μm, membrane thickness ~150 μm) were purchased from Membrana GmbH, Wuppertal. Acrylic Acid (AAc), Benzophenone (BP) and Lysozyme (Lys) were obtained from Fluka. Acrylamide (AAm) and N,N'-methylene bisacrylamide (MBAA) were purchased from Aldrich. The HEPES buffer was from Sigma-Aldrich. Heptane, methanol and NaCl were from Applichem. Sodium hydroxide and Hydrochloric acid were from Waldeck.

2.2 UV photoinitiated grafting

A membrane sample with a diameter of 25 mm was presoaked in 2 ml solution of BP (1 wt.%) in heptane for 1 hour. Then, it was taken out and dried in air for 10 min.. Thereafter, it was wetted in methanol for 5 min., and then wiped with a filter paper before it was put in the monomer solution for 30 min.. In the next step, the membrane was irradiated by using high intensity UV (UV-A Print, Hoenle, Gräfelfing, Germany) and a glass filter (> 300 nm) for 15 min.. Thereafter, it was washed intensively with water before it was dried in an oven for 1 day. Then, the membrane weight was measured, and the degree of functionalization (DG) was calculated using the weight of the original membrane sample and the specific weight (normalized to the outer membrane surface).

2.3 Membrane permeability

An Amicon cell 8010 (Millipore) was used for permeability measurements with water adjusted to pH 2 or pH 10, by adding HCl or NaOH solutions, respectively. Each permeability value was obtained from an average of 5 data which was taken by collecting the filtrate for 30 sec. and determining its amount gravimetrically.

2.4 Membrane chromatography

The liquid chromatograph ÄKTApurifier (Amersham Pharmacia Biotech) was used. 3 membrane samples with a diameter of 12 mm were used as a stack in a CIM® module (BIA Separations, Ljubljana, Slovenia). Buffer A (10 mM HEPES, pH 7.0) was used for membrane equilibration, protein binding and subsequent washing, while buffer B (10 mM HEPES, pH 7.0 + 1 M NaCl) was used for elution. Detection wavelength was 280 nm. The gradient program was as follows: 0-3 min.: flow 1.0 mL/min Buffer A; 3-4 min.: flow 0.5 mL/min Buffer A; 4 min.: sample injection; 4-12 min.: flow 0.5 mL/min Buffer A; 12-16 min.: flow 0.5 mL/min linear gradient to buffer B; 16-19 min.: flow 1.0 mL/min buffer A.; 19 min.: end. A blank gradient was run as the first step, and then two injections of 1 ml solution of Lys (5 mg/mL in buffer A) followed. Calibrations were done by injection of Lys solutions in Buffer A with different concentrations using the CIM® module without membrane stack.

3.0 RESULTS AND DISCUSSION

3.1 Degree of Functionalization

Variations of the cation exchanger group (carboxyl) amounts and the grafted layer crosslinking were attempted by varied monomer composition used for photo-grafting. Taking into account the different monomer reactivities, the total monomer concentration was adjusted in order to obtain a similar degree of grafting, which should then allow investigating the influence of different grafted composition and architecture (Table 1).

Table 1 Degree of functionalization for PP membranes grafted with different polymer layers.

	Monomer concentration in water			Degree of functionalization DG (µg/cm²)	Var. coeff (%)	Number sample
	AAc (g/L)	AAm (g/L)	MBAA(g/L)			
PP-g-PAAc	15	-	-	360±50	13.9	23
PP-g-PAAc-AAm	10	10	-	410±40	10.1	19
PP-g-PAAc-LMBAA	15	-	0.75	390±60	16	19
PP-g-PAAc-HMBAA	12.5	-	1.25	360±40	12	19

The photo-grafting method showed a modest reproducibility, because the variation of the DG values had been always less than 20%. Among the four grafted membrane types, PP-g-AAc-AAm had the highest DG value while PP-g-PAAc and PP-g-PAAc-HMBAA had the lowest DG values. Overall, a rather uniform degree of functionalization had been obtained, with an average of ~380 μg/cm² for all membranes.

3.2 Membrane permeability and its response to pH change

The analysis of membrane permeability can yield information about the blocking of pores by the grafted polymer layers. Furthermore, due to the reversible deprotonation of carboxyl groups above the pK value (pH ~5), significant changes of the effective layer thickness can also be deduced from those data [4,7].

All grafted membranes had a high water permeability during filtration at pH 2 (Fig. 1), and the date were similar to the unmodified PP membrane (11,000 L/hm²bar). The PP-g-PAAc-HMBAA membranes had the highest permeability (13,900 L/hm²bar) while the PP-g-PAAc-AAm membranes showed the lowest values (12,300 L/hm²bar). Hence, all functionalized membranes seemed to have only slightly different effective pore size. The improved water permeability could be explained by the much higher hydrophilicity as compared with PP.

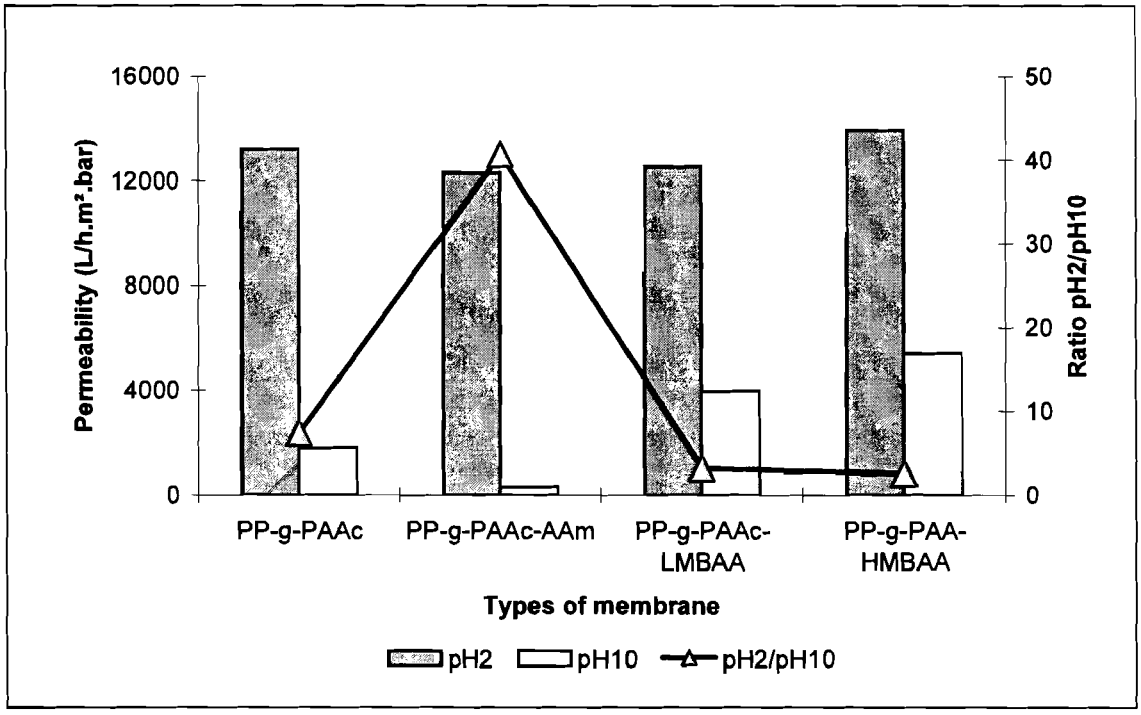

Figure 1 Water permeabilities and the permeability ratio pH 2 vs. pH 10 for membranes with different grafted surface architecture.

The permeability for all grafted membranes reduced significantly during filtration at pH 10. Interestingly, PP-g-PAAc-HMBAA still exhibited the highest permeability with (5,400 L/h.m².bar) while the PP-g-PAAc-AAm membranes showed by far the lowest water permeability (300 L/h.m²bar).

The reversible deprotonation of carboxylic groups in grafted polyacrylic acid segments leads to an ionic repulsion and an osmotic pressure, both forcing the polymer brush to stretch. This phenomenon leads to a decrease of effective membrane pore size, as a result a lower permeability is observed. For the membranes grafted with acrylamide copolymers of AAc, two additional effects should play a role, for PP-g-PAAc-AAm a "dilution" of carboxyl groups, and for the PP-g-PAAc-MBAA membranes the crosslinking of the grafted chains.

The somewhat surprising behaviour of the PP-g-PAAc-AAm membranes can be explained based on a higher (and pH-independent) swelling of the grafted PAAm segments as compared with the PAAc segments. Therefore, at high pH, the overall stretching of the copolymer brush is even larger than for PAAc homopolymer. At low pH, the formation of hydrogen bonds between PAAc and PAAm segments, present in about equal amounts, reduces the swelling below the degree what would have been expected by the contribution of the PAAm segments.

The most interesting finding was that the water permeabilities of the membranes prepared with the bisacrylamide crosslinker were significantly higher, especially at pH 10. Similar to what had been observed with other systems [8], the polymer network can limit the swelling / stretching of the polymer brush layer in the membrane pores, and the permeabilities increases. This effect can be tailored by the content of crosslinker in the monomer mixture.

3.3 Protein binding under membrane chromatography conditions

Reversible protein binding was evaluated using lysozyme, having a molecular weight of 13.9 kDa and an isoelectric point of about 11.9. Hence, at neutral pH, protein binding can occur via cation-exchange with carboxyl groups in the grafted layer on the membrane pores, and elution should be achieved by high salt concentration (Table 2).

Table 2 Reversible protein binding of membranes with different grafted surface architecture (injected lysozyme amount 5.0 mg).

Photo-grafted membrane	No. of injection	Lysozyme bound (mg)	Lysozyme eluted (mg)	Recovery (%)
PP-g-PAAc	1st	3.80	2.91	76
	2nd	3.79	3.03	80
PP-g-PAAc-AAm	1st	3.74	2.78	74
	2nd	3.65	2.89	79
PP-g-PAAc-LMBAA	1st	3.88	3.62	93
	2nd	3.78	3.83	101
PP-g-PAAc-HMBAA	1st	3.26	2.99	92
	2nd	3.22	3.16	98

Rather high amounts of Lys binding were observed for all membranes, corresponding to about 30 mg/ml bed volume. This is in the same range as reported for other membrane adsorbers [1,2], and it can only be explained by a three-dimensional "packing"

of Lys in the grafted polymer layers. However, not in all cases, this amount could be quantitatively recovered in the elution peak.

Focusing on eluted Lys amounts, the membranes with crosslinked grafted layer had higher values, and the AAc-AAm copolymer layers had values similar to the AAc homopolymer. Also, the recovery was significantly higher for the crosslinked AAc-MBAA copolymer membranes; in the second runs ~100% of the bound Lys could be recovered. Overall, an optimum of protein separation performance (highest binding and recovery) had been observed for the PP-g-PAAc-LMBAA membranes, i.e. the grafted layers with a low crosslinker content.

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